

Ecosystem N Distribution and $\delta^{15}\text{N}$ during a Century of Forest Regrowth after Agricultural Abandonment

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ABSTRACT

Stable isotope ratios of terrestrial ecosystem nitrogen (N) pools reflect internal processes and input–output balances. Disturbance generally increases N cycling and loss, yet few studies have examined ecosystem $\delta^{15}\text{N}$ over a disturbance–recovery sequence. We used a chronosequence approach to examine N distribution and $\delta^{15}\text{N}$ during forest regrowth after agricultural abandonment. Site ages ranged from 10 to 115 years, with similar soils, climate, land-use history, and overstory vegetation (white pine *Pinus strobus*). Foliar N and $\delta^{15}\text{N}$ decreased as stands aged, consistent with a progressive tightening of the N cycle during forest regrowth on agricultural lands. Over time, foliar $\delta^{15}\text{N}$ became more negative, indicating increased fractionation along the mineralization–mycorrhizal–plant uptake pathway. Total ecosystem N was constant across the chronosequence, but substantial internal N redistribution occurred from the mineral soil to plants and litter over 115 years (>25% of ecosystem N or

1,610 kg ha⁻¹). Temporal trends in soil $\delta^{15}\text{N}$ generally reflected a redistribution of depleted N from the mineral soil to the developing O horizon. Although plants and soil $\delta^{15}\text{N}$ are coupled over millennial time scales of ecosystem development, our observed divergence between plants and soil suggests that they can be uncoupled during the disturbance–regrowth sequence. The approximate 2‰ decrease in ecosystem $\delta^{15}\text{N}$ over the century scale suggests significant incorporation of atmospheric N, which was not detected by traditional ecosystem N accounting. Consideration of temporal trends and disturbance legacies can improve our understanding of the influence of broader factors such as climate or N deposition on ecosystem N balances and $\delta^{15}\text{N}$.

Key words: $\delta^{15}\text{N}$; soil nitrogen; secondary succession; root biomass; foliar nitrogen; chronosequence; nitrogen isotopes; ecosystem nitrogen; white pine.

INTRODUCTION

Trends in nutrient dynamics over the course of ecosystem development are a fundamental re-

search area in ecology (Vitousek and Reiners 1975; Bormann and Likens 1994; Crews and others 1995). Long-term trends involve many years of N inputs and losses from ecosystems, the balance of which can be reflected in plant and soil $\delta^{15}\text{N}$ (Högberg 1997; Austin and Vitousek 1998). Changes in plant and soil $\delta^{15}\text{N}$ during primary succession have been well studied over time scales from several hundred to several million years (Vitousek and

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others 1989; Hobbie and others 1998; Brenner and others 2001), yet fewer studies examine changes in plant and soil $\delta^{15}\text{N}$ over shorter time scales during the course of secondary succession (Chang and Handley 2000; Pardo and others 2002). Although data are limited, changes in $\delta^{15}\text{N}$ associated with disturbance appear to be of the same magnitude as changes associated with other ecosystem state factors such as climate, vegetation or parent material (Austin and Vitousek 1998; Schuur and Matson 2001; Amundson and others 2002). Ecosystems not in steady-state could reflect disturbance influences on N sources and availability that would obscure the effects of broader-scale factors on the isotope ratios of ecosystem pools.

Over decadal to century scales, redistribution of N within an ecosystem could be more important than ecosystem balances in controlling plant and soil $\delta^{15}\text{N}$. Uptake of N from the soil and conversion to plant biomass and litter is a significant internal redistribution. In forests with low to moderate anthropogenic loading ($<10 \text{ kg N ha}^{-1} \text{ y}^{-1}$), inputs and losses are generally much smaller than plant uptake ($20\text{--}50 \text{ kg N ha}^{-1} \text{ y}^{-1}$; Cole and Rapp 1981). Thus patterns of plant and soil $\delta^{15}\text{N}$ early in succession may be driven by internal redistribution of N from soil to plants rather than ecosystem-level losses. A great deal of the effort examining the $\delta^{15}\text{N}$ of plants has focused on plant processes or plant-symbiont physiology (for example, Michelsen and others 1996; Evans 2001; Hobbie and others 2000), with less emphasis on how plant $\delta^{15}\text{N}$ intersects with changing N availability through ecosystem development. To better use isotope ratios for understanding ecosystem processes, constrained examinations of the patterns of ecosystem N content and $\delta^{15}\text{N}$ over time are needed, particularly over time scales relevant for disturbance and recovery in terrestrial landscapes.

Here we present N content and $\delta^{15}\text{N}$ of plants and soils over the course of forest regrowth after agricultural abandonment. This study uses a chronosequence of white pine stands ranging in age from 10 to 115 years after cultivation ceased (Hooker and Compton 2003). By examining trends in ecosystem N content and isotope ratios over secondary succession, we address several questions: What is the pattern of $\delta^{15}\text{N}$ in plants and soils over the course of agricultural abandonment? Do plants and soils follow the same trend? Is the $\delta^{15}\text{N}$ of ecosystem N pools a more sensitive measure of N dynamics than total pools, which are notoriously variable and difficult to measure? Rooting patterns could influence N availability and isotope ratios, and therefore we present root biomass and distri-

bution for all sites. We also examine the relative importance of N balances versus internal redistribution in driving the changes in ecosystem $\delta^{15}\text{N}$ over time.

METHODS

Study Area and Site Selection

The study area is located within the Scituate watershed, Rhode Island, USA. The climate is temperate, with a mean annual temperature of 9.1°C and a mean monthly maximum of 21.1°C and minimum of -3.7°C . Annual precipitation is 128 cm (range between 1915 and 2003 is 85–171 cm; <http://www.provwater.com>). Approximately 88% of the basin is forested, dominated by mixed oaks (*Quercus velutina*, *Q. alba*, *Q. rubra*, and *Q. coccinea*), and conifers [primarily white pine (*Pinus strobus*), red pine (*P. rigida*) or eastern juniper (*Juniperus virginiana*)]. The soils are well drained, glacial till-derived soils classified as the Canton and Charlton series complex, which are coarse-loamy over sandy or sandy-skeletal, mixed, mesic Typic Dystrochrepts (Rector 1981). Wet plus dry inputs from atmospheric deposition are estimated to be $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ (Ollinger and others 1993).

During the early 1900s, the city of Providence obtained much of the Scituate watershed for creation of a drinking water reservoir. Town populations declined from 1850 until the 1930s, when they increased as suburban areas developed around Providence. Land use from 1850 to 1890 was predominantly dairy and cattle operations that produced hay and grazed cattle in a 2–3-year rotation. Interviews with the owners of recently abandoned farms did not reveal use of mineral fertilizers.

Site descriptions are shown in Table 1. Potential sites were identified using 1916–1917 land-use maps, in conjunction with land cover, soils and digital orthophotos to select areas with similar soils and vegetation. All sites are within 5 km of each other. Site selection was based on the following criteria: evidence of previous cultivation from 1916–1917 maps, overstory dominated by white pine, Canton–Charlton soil series complex, slope of 8% or less, and field evidence of a plow layer (Ap horizon). We excluded sites that had been thinned during the last 30 years. We also sampled two uncultivated but otherwise similar sites; these sites were used for pasture but allowed to revert to white pine approximately 63 and 101 years ago. We used aerial photographs, increment boring and air photo interpretation to determine the time since abandonment or “site age” relative to 1998.

Table 1. Description of Chronosequence Site Age, and Overstory Basal Area and Aboveground Biomass (AGBM) in Scituate Watershed, Rhode Island

Site age ¹	Date of abandonment	Basal area (m ² ha ⁻¹)	AGBM (Mg ha ⁻¹)
10	1988	0.1	8.9
16	1982	2.3	13.1
23	1975	2.8	23.7
23	1975	2.2	13.9
47	1951	26.0	66.9
53	1945	14.1	52.0
72	1926	47.8	167.2
99	1899	38.9	184.6
99	1899	59.4	302.5
114	1884	51.9	249.8

From Hooker and Compton 2003.

¹ Time since abandonment of cultivation.

Aboveground Biomass and Litterfall

Aboveground biomass and N content measurements are presented by Hooker and Compton (2003). Briefly, measurements were taken within a 30 × 30-m plot located in the center of each site. Diameter and height were measured on all trees above 5 cm diameter. At each site, stemwood and bark were collected using two increment cores from two trees per dominant tree species. For stands less than 60 years old, branches from the upper and lower third of white pine and juniper canopy trees were collected using a pole pruner from at least two trees per species per site. For stands older than 60 years, white pine branch and leaf material was collected within ten days after Tropical Storm Floyd passed over Rhode Island (17 September 1999). Leaf N concentrations were not significantly different between samples collected 1 day versus 10 days after the storm. At least ten branch samples were composited at each site; needles from all ages were collected (age 1 and 2 years for white pine, undetermined for juniper). The samples were collected from a number of locations ($n \geq 10$), to integrate the variability within the site (Garten and Van Miegroet 1994). Understory aboveground biomass was destructively sampled from five 0.5-m² quadrats per site, and separated into woody and non-woody material. Overstory litterfall was estimated from biweekly collections from four collectors (50 × 24 cm) per site from October through November, 1998.

Plant tissue and litterfall were dried at 70°C, ground and analyzed by Carlo-Erba NA1500

Carbon–Nitrogen analyzer (Carlo-Erba Instruments, Milan, Italy). Leaf, branch and bole N concentrations were multiplied by tree component biomass from allometric equations (see Hooker and Compton 2003), and summed for each tree and site to calculate stand-level aboveground biomass N content. For understory biomass, N concentrations of woody and foliage material were multiplied by the component mass to calculate N contents, and added to the overstory data for total aboveground biomass per plot.

Soil and Root Measurements

We sampled the soil using a quantitative pit method described in detail by Hooker and Compton (2003). Briefly, three soil pits (50 × 50 cm) were excavated at each site, with samples taken at horizon boundaries for O and A horizons and at pre-determined depth increments within the Ap and B horizons to 70 cm depth. All soil was weighed, and volume determined using a 25-point grid. Compact basal till occurred near 70 cm in most of the profiles, which suggests that the sampling depth represents the entire solum.

Soil samples were sieved (<2 mm), and the 2 mm and above fraction was sorted into gravel and roots. Roots were rinsed with deionized water on a 0.5 mm sieve, dried at 70°C, separated into coarse (>3 mm) and fine (<3 mm) roots and weighed. Root N concentration was determined only on the composite of fine plus coarse roots. Coarse root biomass likely is underestimated because we did not excavate whole tree root systems. Mineral soils, forest floor and root samples were ground and dried at 70°C before analysis for C and N. We observed less than 5% analytical variability in random duplicate samples for N. Soil and root pools for all horizons were summed for each soil pit, and then pits were averaged within a site to calculate C and N content. Regressions were conducted using SYSTAT version 10 (SPSS, Inc., Chicago, Illinois, USA).

Nitrogen Isotope Analyses and Modeling

Foliage, O horizon and mineral soils (0–20 cm depths) were analyzed for N isotope ratios using a continuous flow isotope ratio mass spectrometer (Finnigan MAT Delta Plus XL, Thermo-Finnigan, Watertown Massachusetts, USA) at EPA's Integrated Stable Isotope Research Facility in Corvallis, Oregon, USA. Soil samples collected from the greater than 20 cm depth contained too little N to determine isotope ratios at that time. The standard deviation of replicate analyses of foliage and soil

$\delta^{15}\text{N}$ was less than 0.1‰. The mean value for the NIST Montana soil #2710 was 4.85‰ with a SD of 0.12‰. A value of 4.93‰ for this standard was obtained by long-term analyses of this sample. The discrimination factor of plants relative to soil (Garten and Van Miegroet 1994) was calculated as foliar $\delta^{15}\text{N}$ minus the N mass-weighted soil $\delta^{15}\text{N}$ (0–20 cm mineral). We calculated mass-weighted soil $\delta^{15}\text{N}$ by summing the products of soil N by soil $\delta^{15}\text{N}$ for each horizon, then dividing by the total N mass.

We modified the modeling approach of Perakis and Kellogg (2007) to examine our assumptions about the influence of N transfers on soil, plant and ecosystem $\delta^{15}\text{N}$. We also use this approach to estimate the $\delta^{15}\text{N}$ value of N losses, because these are difficult to measure, but important for understanding ecosystem N balances and $\delta^{15}\text{N}$. Our modified model has two pools consisting of plants and soil (O horizon plus mineral soils to 20 cm), whose N and $\delta^{15}\text{N}$ dynamics were constrained by linear regression trends in N and $\delta^{15}\text{N}$ measured in the field (see Results). Soil was initialized using an N pool ($3,519 \text{ kg N ha}^{-1}$) and $\delta^{15}\text{N}$ (4.92‰) estimated from the intercept of the trends over time in soil N and $\delta^{15}\text{N}$ (O horizon plus 0–20 cm mineral soil). The plant N pool started at zero, and accumulated N from soil at a rate ($4.13 \text{ kg N ha}^{-1} \text{ y}^{-1}$) indicated by field data. Plant $\delta^{15}\text{N}$ was calculated relative to soil $\delta^{15}\text{N}$ using the linear regression of plant–soil discrimination over succession. Atmospheric deposition was the only input considered, at a rate of $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ (Ollinger and others 1993) and $\delta^{15}\text{N}$ value of -3‰ (based on values for Hubbard Brook, NH, from Pardo and others 2004). Ecosystem N losses were considered to originate from soil as a single pathway representing all losses (hydrologic and gaseous), and set at a rate to balance N inputs, based on field data that show no discernable change in ecosystem N over succession. With the model constrained by these empirical and literature data, we then asked what $\delta^{15}\text{N}$ of losses (relative to soil $\delta^{15}\text{N}$) was necessary to reproduce the field trend in whole-ecosystem $\delta^{15}\text{N}$ observed over succession.

RESULTS

Patterns in N Content and Distribution

Total ecosystem N did not vary significantly over time, but distribution of N within the ecosystem did change (Figure 1). Plant uptake from the mineral soil and accumulation of litter in the O horizon drove this redistribution. Plant biomass N repre-

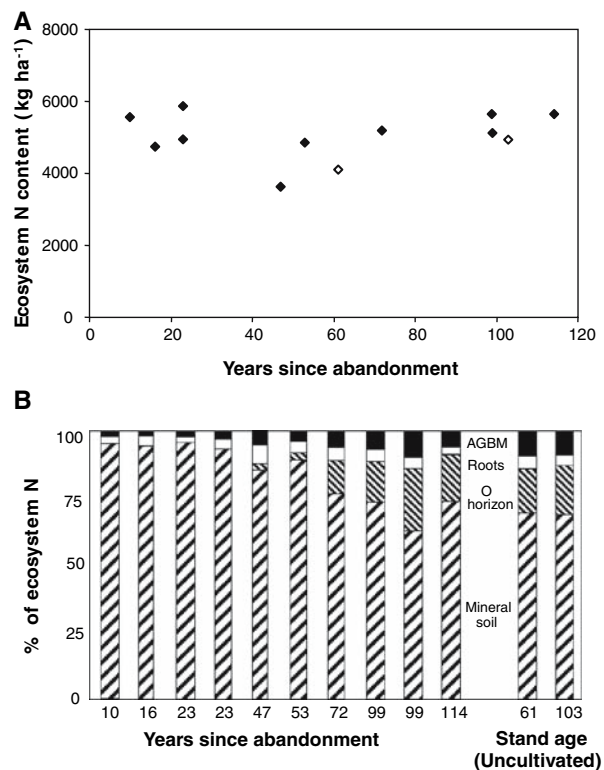


Figure 1. Ecosystem N content **A** and distribution **B** over time to 70 cm soil depth. Rate of increase of ecosystem N ($3.3 \text{ kg N ha}^{-1} \text{ y}^{-1}$) is not significantly different from zero. Open symbols in **A** represent uncultivated sites. AGBM, aboveground biomass.

sented 5% of total ecosystem N early in succession, then increased to approximately 10–15% of ecosystem N in the century-old stands. Belowground biomass N was approximately one-third of total plant biomass N in the oldest sites. Litterfall N increased rapidly during the first 50 years of stand development, then remained relatively constant (Figure 2A). The O horizon was not present in sites younger than 47 years, but rapidly increased to approximately 20% of ecosystem N in the oldest stands.

Nitrogen concentrations in white pine foliage decreased significantly over time, from approximately 2.0% N in the youngest stands to 1.5% N in the oldest stands (Figure 2B). Eastern juniper had slightly lower N concentrations than pine, and did not vary with time ($R^2 = 0.40$; not significant). Juniper is an early successional species, and was not found in sites older than 60 years. Because stand level leaf mass is fairly constant (data not shown), a decrease in pine foliar %N suggests that N availability declined with time.

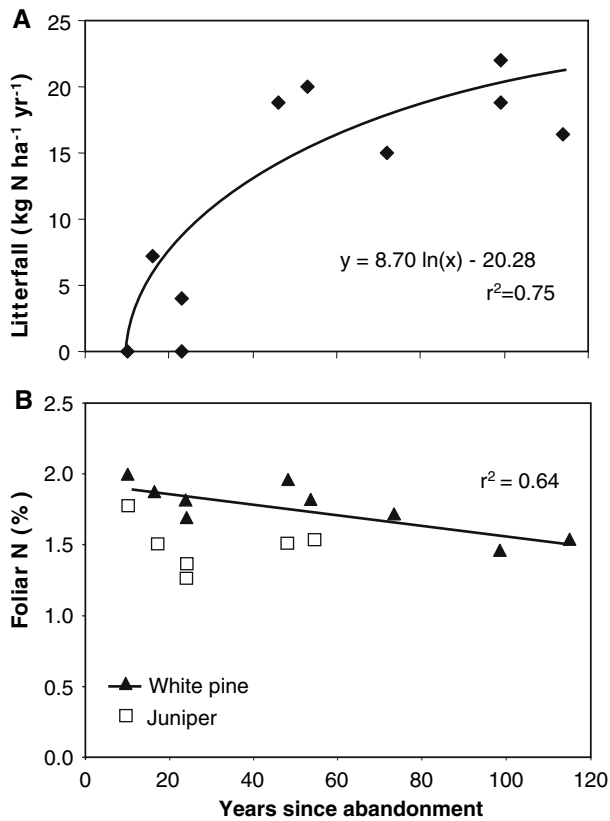


Figure 2. Overstory **A** litterfall rate and **B** foliar %N across the chronosequence. Significant regressions are shown ($P < 0.05$).

Root Biomass

Root biomass increased with time since abandonment (Table 2). Total root biomass increased in the forest floor and subsoil over time, but remained constant in the former plow layer (Table 2; Figure 3). Approximately 60% of the increase in root biomass over time occurred below 20 cm (Table 2). In the youngest sites, fine roots dominated the total root biomass (>95%). The biomass of fine (<3 mm) roots doubled across the chronosequence (4.0–8.6 Mg C ha⁻¹).

Coarse root (>3 mm) biomass in the soil profile increased significantly over time (Table 2) from approximately 0.6 to 6.8 Mg C ha⁻¹, and came to represent approximately 45% of root biomass in the oldest sites. Coarse root biomass was highly variable within all sites and sample depths [average coefficient of variation (cv) = 110%] as compared to fine root biomass (average cv = 41%). Although 46% of all soil samples (96 out of 210) had no coarse roots, the occasional presence of very large coarse roots (>20 mm in diameter) in some samples drove this variability.

In contrast to total root biomass, total root N was not related to site age for the entire profile (Table 2). However, there were significant increases in root N content within the subsoil horizons (20–40, 40–55, 55–70 cm), driven by increases in root biomass in those horizons.

Foliar, Soil and Ecosystem $\delta^{15}\text{N}$

The $\delta^{15}\text{N}$ of white pine foliage was relatively constant during the first 60 years (values between 2 and 3‰), then declined to between 0 and –2‰ thereafter (Figure 4A). Juniper was observed only in sites younger than 60 years; juniper foliage was less than 0‰, and did not change over time. White pine foliar N and $\delta^{15}\text{N}$ were positively correlated ($R^2 = 0.65$). Pine foliar $\delta^{15}\text{N}$ was the same or slightly lower in the uncultivated sites as compared to similar aged cultivated sites (–2.39 and –1.42‰ in 63 and 101-year-old stands, respectively). For each site, averages of ecosystem pool N and $\delta^{15}\text{N}$ are shown in Appendix 1 (<http://www.springer-link.com>).

Patterns in the $\delta^{15}\text{N}$ of the O horizons depended on the decomposition state. The $\delta^{15}\text{N}$ of the relatively undecomposed Oi horizon was similar to white pine foliage and also declined over time (Figure 4). In contrast, $\delta^{15}\text{N}$ of the more decomposed Oe horizon increased steadily over time, comparable to the pattern in surface mineral soil. Mixing of mineral soil during sampling or by root activity into the Oe horizon could increase in $\delta^{15}\text{N}$ because mineral soils have higher $\delta^{15}\text{N}$. However, %C of the Oe horizon increased over time, suggesting that mixing of mineral material into the horizon did not influence the $\delta^{15}\text{N}$.

Mineral soil $\delta^{15}\text{N}$ increased with time since abandonment (Figure 4B). Mineral soil $\delta^{15}\text{N}$ also increased with depth, and the range of $\delta^{15}\text{N}$ increased with stand age (Figure 5). Surface horizons showed the strongest increases with time, and the rate of $\delta^{15}\text{N}$ enrichment declined with depth in the mineral soil. Linear increases in isotope ratios ranged from 2.1 and 1.1‰ per century in the Oe and Ap2 horizons, respectively (Table 3).

Ecosystem components were combined and weighted by N pool size to determine the $\delta^{15}\text{N}$ of aggregate soils and the ecosystem over time (Figure 6A). The ecosystem $\delta^{15}\text{N}$ could be assessed only to 20 cm soil depth due to analytical limits. We also assumed that foliar $\delta^{15}\text{N}$ represents the entire plant pool. Aggregate ecosystem $\delta^{15}\text{N}$ decreased about 1.7‰ over time, reflecting an increase in the ^{15}N -depleted plant N pool over time (Figures 1, 4). Model results are shown in Figure 6C. We explored

Table 2. Linear Regressions of Root Biomass and N Content against Time since Abandonment

Horizon	Fine root biomass (Mg ha ⁻¹ y ⁻¹)			Coarse root biomass (Mg ha ⁻¹ y ⁻¹)			Total root biomass (Mg ha ⁻¹ y ⁻¹)			Total root nitrogen (kg ha ⁻¹ y ⁻¹)		
	R ²	Slope	P	R ²	Slope	P	R ²	Slope	P	R ²	Slope	P
Oe	0.28	0.04	*	0.13	0.01	ns	0.25	0.05	*	0.21	0.42	ns
Ap 0–20 cm	0.02	–0.01	ns	0.11	0.08	ns	0.06	0.07	ns	0.02	–0.24	ns
20–40 cm	0.63	0.04	***	0.07	0.08	ns	0.13	0.18	*	0.17	0.38	*
40–55 cm	0.56	0.03	***	0.06	0.03	ns	0.18	0.05	*	0.22	0.21	**
55–70 cm	0.46	0.03	***	0.09	0.00	ns	0.46	0.03	***	0.37	0.14	***
20–70 cm	0.65	0.09	***	0.07	0.11	ns	0.19	0.20	*	0.27	0.74	**
Solum	0.44	0.11	***	0.14	0.19	*	0.26	0.30	**	0.09	0.77	ns

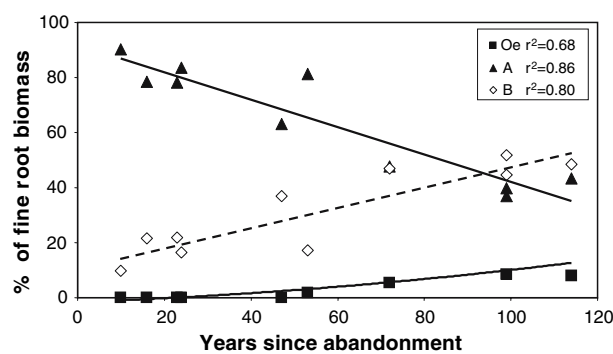


Figure 3. Distribution of fine root biomass in each major soil horizon over time. A horizon represents the sum of A, Ap1 and Ap2 horizons (0–20 cm depths). B Horizon is the sum of Bw1 and Bw2 horizons (20–70 cm depths).

a range of fractionation factors associated with N losses in the model, seeking to reproduce the 1.7‰ decrease in ecosystem $\delta^{15}\text{N}$ over time shown in Figure 6A using known information on plant, soil and deposition N and $\delta^{15}\text{N}$. When modeled N losses were 10‰ more depleted than soil N (that is, typical of nitrate leaching), simulated ecosystem $\delta^{15}\text{N}$ increased slightly over time (dashed line in Figure 6C), at odds with field observations. The solid line in Figure 6C depicts model output that most closely resembles observed trends in ecosystem $\delta^{15}\text{N}$, and resulted from setting N losses at approximately 0.8‰ depleted relative to soil N.

DISCUSSION

Trends in Foliar $\delta^{15}\text{N}$ Over Time: Role of Plant N Sources and Fractionation

Foliar $\delta^{15}\text{N}$ decreased at a faster rate than soil $\delta^{15}\text{N}$ with time since disturbance over our chronosequence. Initially, the discrimination between plants and soil (aggregated to 20 cm) was -2‰ , but

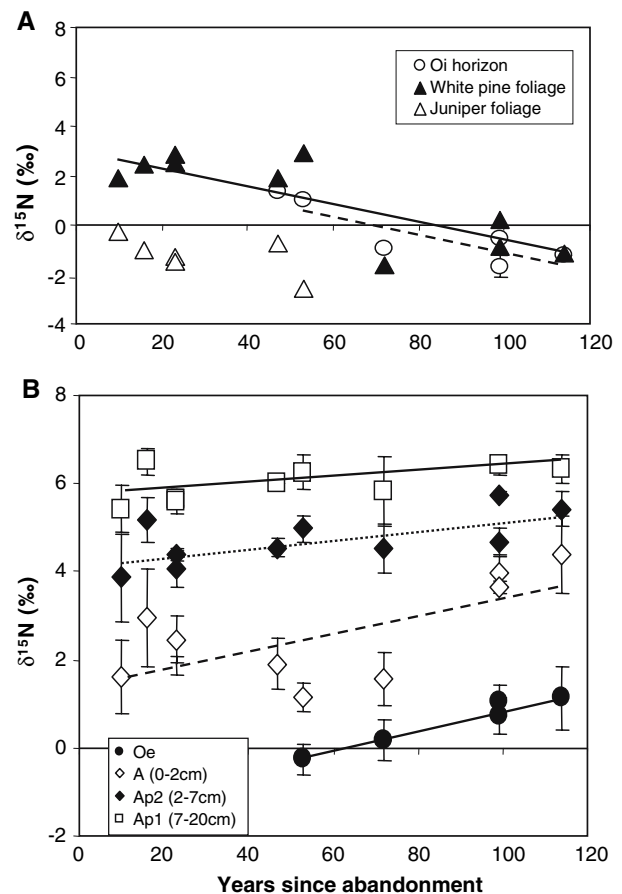


Figure 4. The $\delta^{15}\text{N}$ over time of **A** foliage and Oi horizon and Oe horizon and **B** mineral soil. Only significant regression lines are shown ($P < 0.05$; Table 3).

became more negative over time (Figure 6B), revealing a strong divergence between plants and soil. Although they may be coupled over long time scales of ecosystem development (Austin and Vitousek 1998), our observed divergence between plants and soil suggests that they can be uncoupled

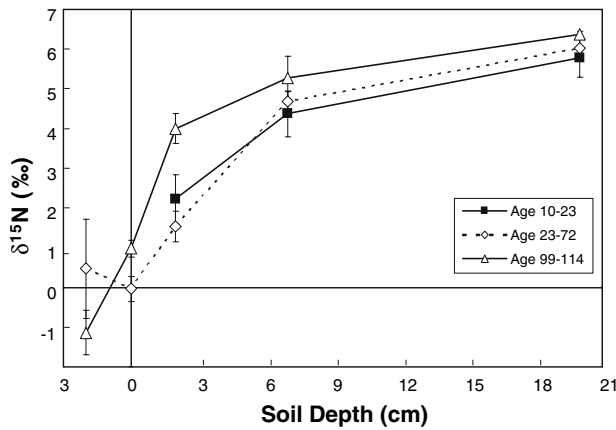


Figure 5. Soil $\delta^{15}\text{N}$ as a function of depth for all sites. Values are averages for all sites within each time period.

Table 3. Linear Regressions of the $\delta^{15}\text{N}$ in Ecosystem Pools against Time since Abandonment

Ecosystem Pool $\delta^{15}\text{N}$	Intercept	Slope	R^2	P
White pine foliage	3.00	-0.040	0.56	0.005
Juniper foliage	-0.42	-0.029	0.40	ns
Oi horizon	1.86	-0.031	0.44	0.07
Oe horizon	-1.33	0.021	0.86	0.002
A horizon (0–2 cm)	1.44	0.021	0.51	0.009
Ap1 horizon (2–7 cm)	4.12	0.012	0.50	0.01
Ap2 horizon (7–20 cm)	5.57	0.011	0.30	0.05

during the disturbance-regrowth sequence. We consider several explanations for the decline in pine foliar $\delta^{15}\text{N}$ over time, including: (1) shifts in root uptake to ^{15}N -depleted surface horizons; (2) fractionation of plant N during uptake or within the plant, including mycorrhizal influences; and (3) changes in soil N cycling rates and losses over time that influence the $\delta^{15}\text{N}$ of available N. A change in the amount or $\delta^{15}\text{N}$ of inputs over time is not considered to be an important factor, because no symbiotic N-fixers were observed, and the inputs from free-living N-fixers and N deposition was expected to be similar across sites (albeit deposition might increase at different points in succession, see last section).

A shift in root distribution or uptake from enriched mineral horizons to depleted surface horizons is one potential explanation for the temporal patterns in foliar $\delta^{15}\text{N}$. It is thought that plants assimilate nutrients in proportion to the depth distribution of roots in soil (Woodward and Osborne 2000). Because soil $\delta^{15}\text{N}$ increases with depth, changes in root distribution and foraging depths could affect the isotope ratio of plant uptake

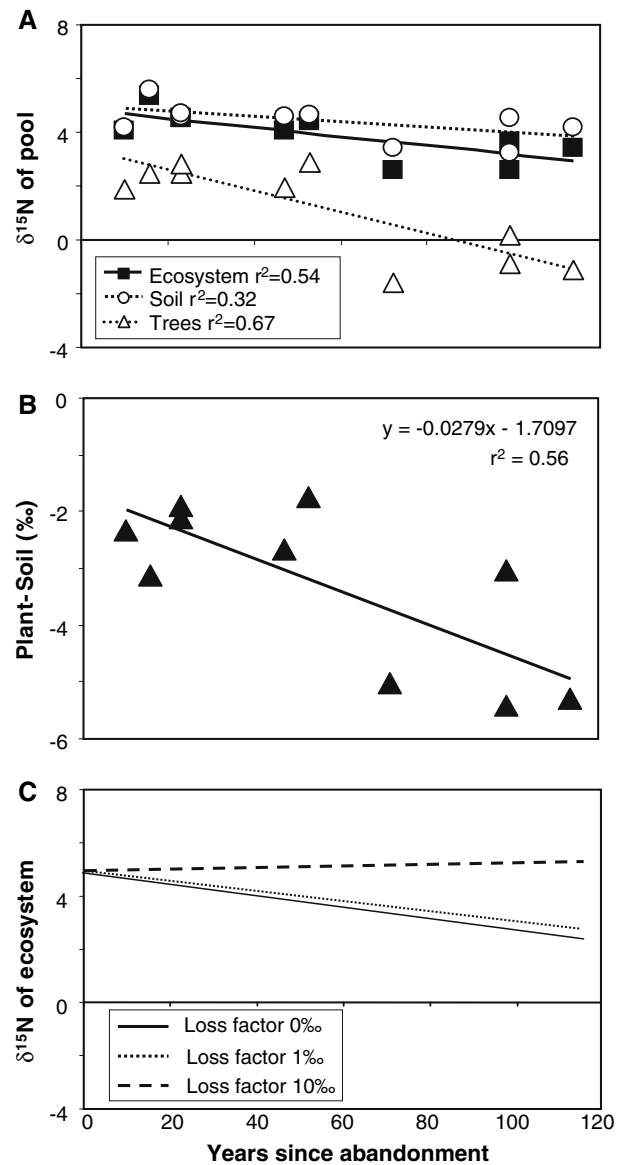


Figure 6. **A** Mass-weighted $\delta^{15}\text{N}$ of ecosystem pools, **B** the discrimination factor between plants and soils, and **C** model results for the whole ecosystem $\delta^{15}\text{N}$ using different values for the difference in $\delta^{15}\text{N}$ between losses and soil organic N. Soil pool includes the O horizon and mineral soil to 20 cm soil depth. Loss factor refers to the ^{15}N depletion associated with losses relative to mineral soil.

(Schulze and others 1994). Fine roots were concentrated in the upper mineral horizons early in succession, but over time root allocation shifted both upward into the developing O horizon and down into the B horizon (Figure 3). To evaluate the effect of this shift, we calculated foliar $\delta^{15}\text{N}$ based on the proportion of roots in each horizon multiplied by the $\delta^{15}\text{N}$ of the soil from each horizon. Values of soil $\delta^{15}\text{N}$ below 20 cm were

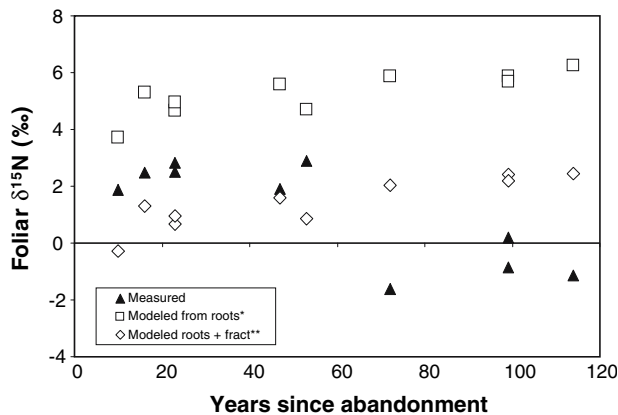


Figure 7. Calculated values of foliar $\delta^{15}\text{N}$ based on root distributions. Measured values shown for comparison. *Weighted by rooting distribution, based on the proportion of the roots in each horizon (from Figure 3) multiplied by the $\delta^{15}\text{N}$ of the soil from each horizon. **Weighted by rooting distribution, plus a time-constant uptake fractionation of 0.5‰ for organic soils and 4‰ for mineral soils.

estimated arbitrarily by adding 1‰ to the value at 7–20 cm. We also considered effects of depth-dependent $\delta^{15}\text{N}$ fractionation from soil to roots, assuming 0.5‰ depletion in $\delta^{15}\text{N}$ of roots relative to soil in the organic horizon, but the 4‰ depletion in mineral horizons (Högberg and others 1996) over the full course of succession. In both cases the calculated foliar $\delta^{15}\text{N}$ increased with time, whereas our field data shows a decrease over succession (Figure 7). Thus changes in root distribution alone cannot explain the decrease in foliar $\delta^{15}\text{N}$.

Fractionation along the mineralization–mycorrhizal–plant uptake process is important to interpreting N isotope patterns in forests. Plant $\delta^{15}\text{N}$ can reflect isotope fractionation upon uptake and assimilation, particularly when N is abundant relative to demands (Evans 2001), but we expect that such fractionation is negligible at our sites as it is in many N-poor forest settings (Högberg and others 1999). Fractionation due to mycorrhizal associations may be important in determining the $\delta^{15}\text{N}$ of plants, owing to mycorrhizal use of different N sources (Michelsen and others 1996; Schulze and others 1994) and fractionation of N transferred to plants (Högberg and others 1999; Hobbie and others 2005). The difference observed in white pine versus juniper foliage $\delta^{15}\text{N}$ (Figure 4) may be a consequence of mycorrhizal associations, because pine form ectomycorrhizae (ECM), whereas junipers form vesicular–arbuscular mycorrhizae (VAM; Smith and Read 1997). Although ECM pine was more enriched than VAM juniper where they

co-occurred at our sites, this is opposite of patterns found in the arctic (Michelsen and others 1996; Emmerton and others 2001), which indicates that the type of mycorrhizal association may influence foliar $\delta^{15}\text{N}$ even if the direction of the shift is not constant.

Successional effects of mycorrhizae and plant available N on foliar $\delta^{15}\text{N}$ is of particular interest in our work. Mycorrhizal fractionation increases as N becomes more limiting, providing progressively more depleted N to plants under N-poor conditions (Hobbie and Colpaert 2003). We observed a decline in foliar %N (Figure 2B) and foliar biomass N over time (data not shown) indicative of a tightening of N availability over succession. Others have seen similar declines in available N pools decline during conifer forest regrowth after agricultural abandonment, due to substantial transfer of mineralized N from the soil to plants (Richter and others 2000). Such a successional decline in N availability could be associated with greater mycorrhizal fractionation of plant available N, and could explain the decline in foliar $\delta^{15}\text{N}$ across our chronosequence. Shifts in mycorrhizal colonization may also occur over succession (Visser 1995), but the functional impact of these shifts is unclear. The effect of decreasing soil N availability on the $\delta^{15}\text{N}$ of ammonium as a preferred N source in conifers (Kronzucker and others 1997) could also influence successional patterns in plant $\delta^{15}\text{N}$ (Högberg 1997). In our study, higher nitrification rates immediately after agricultural abandonment likely increased the $\delta^{15}\text{N}$ of plant-available ammonium in young stands. Potential nitrification (using the method of Schmidt and Belser 1994) was approximately two orders of magnitude greater in the youngest site as compared to the oldest site in our chronosequence (J. E. Compton, unpublished data). Because patterns in potential nitrification correspond to patterns of field net nitrification in similar ecosystems (Compton and Boone 2000), this suggests that nitrification is higher in the young stands. Nitrification would enrich the remaining ammonium taken up by conifers (Högberg 1997), thus yielding the high values of foliar $\delta^{15}\text{N}$ in the youngest stands. Pardo and others (2002) observed a rapid increase in foliar $\delta^{15}\text{N}$ after the disturbance associated with clearcutting, and suggested that the increased nitrification responsible for elevated stream-water nitrate also increased the $\delta^{15}\text{N}$ of plant-available ammonium. Thus the legacy of farming would be reflected in the $\delta^{15}\text{N}$ of the plants early in succession, as we observed here.

Overall, our pattern of declining foliar $\delta^{15}\text{N}$ over succession is consistent with a disturbance legacy of

high nitrification rates immediately after agricultural abandonment. Over time, foliar $\delta^{15}\text{N}$ became more negative, indicating a fractionation along the mineralization–mycorrhizal–plant uptake pathway. Figure 6B suggests a fractionation of -4 to -5‰ after stand age 60. This discrimination is well within the magnitude expected from nitrification and mycorrhizal-dependent fractionation (Högberg 1997; Högberg and others 1999; Hobbie and others 2005).

Increases in Mineral Soil $\delta^{15}\text{N}$ Over Time

Fractionation associated with the microbial process of mineralization of organic N to ammonium is thought to be near zero (Högberg 1997), and nitrification and denitrification rates should be very low in well-drained acid forest soils (Magill and others 1997). However, our study and others illustrate that enrichment of the remaining soil organic N does occur over the timescale of decades to centuries. Mineral soil $\delta^{15}\text{N}$ increased at the rate of approximately $1\text{--}2\text{‰}$ per century, with the greatest increases occurring near the surface. Billings and Richter (2005) also observed an increase in mineral soil $\delta^{15}\text{N}$ over a 40-year-period, through repeated sampling of pine forests in North Carolina.

Fractionating losses of N from soil organic matter occur via many pathways. Because rates of plant N uptake and litter N accumulation are generally greater than hydrologic export, we expect that internal redistributions, rather than input–output balances, drive the pattern of soil $\delta^{15}\text{N}$ at the century timescale. Plant uptake plus litter accumulation was approximately $15.7 \text{ kg N ha}^{-1} \text{ y}^{-1}$ or approximately $1,800 \text{ kg N ha}^{-1}$ over the 115-year-chronosequence (Hooker and Compton 2003), which represents approximately 25% of ecosystem N. Foliar $\delta^{15}\text{N}$ declined over time, and thus can at least partially explain the increase in mineral soil $\delta^{15}\text{N}$ through transfer of ^{15}N -depleted N into the plant biomass. Leaching of inorganic N and DON from the rooting zone averaged less than 0.1 and $5.4 \text{ kg N ha}^{-1} \text{ y}^{-1}$ respectively for a red pine forest in southern New England (Currie and others 1996). Rates of downward migration of DON from the O horizon to the mineral soil ($4.2 \text{ kg N ha}^{-1} \text{ y}^{-1}$; Currie and others 1996) are similar to DON leaching, but it is not clear whether sorption of DON is a fractionating process. Microbial products enriched in ^{15}N may be preferentially stabilized within mineral-associated soil organic matter, because mineral-associated N is consistently enriched relative to organic soil N fractions (Compton and Boone 2000; Baisden and others 2002; Sollins and

others 2006). These findings suggest that in addition to microbial processes, mineral–organic interactions key to the formation of soil organic N contribute to its enrichment in ^{15}N over time. Future work is needed to determine the relative importance and fractionation associated with many processes leading to formation of soil organic N, including microbial turnover, mycorrhizal uptake and turnover, formation of dissolved organic N, mineralization, and sorption and other mineral–organic matter interactions.

Development of the Depth Pattern in Soil $\delta^{15}\text{N}$ Over Time

The pattern of increasing $\delta^{15}\text{N}$ with depth is a common trend in soils (Nadelhoffer and Fry 1988; Piccolo and others 1996). Our data on soil profile $\delta^{15}\text{N}$ over a century of forest regrowth illustrates the development of this depth pattern over time, where the span in $\delta^{15}\text{N}$ with depth increases with stand age (Figure 5). Over time, the pattern is accentuated, because the surface horizons became more depleted and the deeper horizons became more enriched (Figure 5). Farming would have removed ^{15}N -depleted surface horizons and homogenized the surface profile to the plow depth. Over time ^{15}N -depleted organic matter accumulated on the soil surface through plant uptake and litterfall. The $\delta^{15}\text{N}$ reflects the foliage in the Oi horizon (decreasing 3‰ per century; Figure 4A), but then as the litter decomposes to form the Oe horizon, the $\delta^{15}\text{N}$ increases (2‰ per century; Figure 4B). Litterbag decomposition studies also show net increase in $\delta^{15}\text{N}$ in plant materials over the course of several years (Melillo and others 1989; Connin and others 2001), indicating that microbial processes result in a loss of depleted N. The shift in the depth profile shown in Figure 5 follows the pattern hypothesized by Högberg (1997) in forest soil $\delta^{15}\text{N}$ after disturbance.

Examination of the relationship between N content and $\delta^{15}\text{N}$ of a given horizon should provide insight into the extent of these loss processes. In our study, all mineral horizons showed a significant increase in $\delta^{15}\text{N}$, and the maximum increase in mineral soil $\delta^{15}\text{N}$ over time occurred in the $0\text{--}2 \text{ cm}$ horizon (Figure 4). The A and Ap horizons lost approximately $14 \text{ kg N ha}^{-1} \text{ y}^{-1}$ or $1,610 \text{ kg N ha}^{-1}$ across the 115-year-chronosequence whereas the subsoil showed a non-significant gain in N over time of $1.6 \text{ g kg N ha}^{-1} \text{ y}^{-1}$ (Hooker and Compton 2003). In a study using archived samples collected from the Calhoun forest over a 40-year-period, Billings and Richter (2005) found that surface soil

$\delta^{15}\text{N}$ did not change very much whereas the deep soil (35–60 cm) exhibited the greatest change in $\delta^{15}\text{N}$ (9‰). The deep soil also experienced the greatest N losses through the profile, decreasing by about 43% over the 40-year-period (Richter and others 2000). These findings collectively suggest that N loss from a particular horizon is an important factor in the $\delta^{15}\text{N}$ of that horizon.

The influence of losses of N from a soil horizon on the $\delta^{15}\text{N}$ of the remaining soil N pool can be quantified, to provide some bounds on the fractionation needed to explain the losses. The net fractionating effect of all N transformations in the former plow horizon 0–20 cm was calculated using the Rayleigh equation ($\delta^{15}N_{\text{final}} = \delta^{15}N_{\text{initial}} + \epsilon \times \ln(N_{\text{initial}}/N_{\text{final}})$), where ϵ is the fractionation factor and N mass for the 0–20 cm depth is kg N ha^{-1} . The fractionation factor was -3.0‰ , which is substantially lower than the value of 9.7‰ from Billings and Richter (2005), but still within the range observed for nitrification and mycorrhizal fractionation (Högberg 1997). An underlying assumption of the Rayleigh approach is a closed system, and thus losses from the pool are the only cause of fractionation. However, root N increased over time in the deeper mineral soils (Table 2), revealing a potential addition of depleted N to mineral soil from root turnover as well, which may explain why the values for ϵ and fractionation factor are relatively low. The assumption of no N additions to soils (for example, from root turnover, DON migration or incorporation of atmospheric N) is most likely not met, perhaps limiting the application of a Rayleigh-type equation in open systems such as these regrowing forests.

Trends in Ecosystem $\delta^{15}\text{N}$ with Forest Age

The balance between N isotope ratios of inputs and losses produces characteristic patterns in $\delta^{15}\text{N}$ of ecosystem pools (Austin and Vitousek 1998; Houlton and others 2006). Without disturbance, fractionating losses such as nitrate leaching and denitrification cause ecosystems to become N enriched in $\delta^{15}\text{N}$ over long time scales, given constant inputs. Enrichment in soil $\delta^{15}\text{N}$ over time means that plant $\delta^{15}\text{N}$ also should increase over long time scales in the absence of disturbance (Vitousek and others 1989). However, disturbances such as fire, cultivation and logging have the potential to increase ecosystem $\delta^{15}\text{N}$ by fostering pulsed losses of ^{15}N -depleted plant biomass, litter, nitrous oxide and nitrate. Patterns of ecosystem ^{15}N then may be complicated in years immediately following disturbances, with ^{15}N increasing initially due to a

pulse of fractionating losses, then decreasing immediately thereafter as biological recovery tightens the N cycle and reduces N losses (Pardo and others 2002). We observed that plant $\delta^{15}\text{N}$ decreased over time as mineral soil $\delta^{15}\text{N}$ increased during the same time period in early succession. Although long-term ecosystem trends over thousands to millions of years allow soils and plants to be closely coupled, shorter-term patterns reveal an uncoupling of soil and plant $\delta^{15}\text{N}$ shown in our study.

Examination of ecosystem $\delta^{15}\text{N}$ may reveal trends in N accumulation not measurable by conventional ecosystem N accounting approaches. Although there was no detectable change in total ecosystem N pools over a century of forest regrowth (Hooker and Compton 2003), ecosystem $\delta^{15}\text{N}$ decreased from about $+5$ to $+3\text{‰}$. Measurement of soil N pools, in particular, can have large uncertainties (Binkley and others 2000; Homann and others 2001). The decrease in ecosystem $\delta^{15}\text{N}$ over time suggests that the system is in an N accumulation phase, because ecosystem $\delta^{15}\text{N}$ is approaching deposition (-6 to 0‰ ; Pardo and others 2004) and N_2 -fixation (Shearer and Kohl 1986). If net losses of N from the ecosystem were occurring as nitrate or N_2O , ecosystem $\delta^{15}\text{N}$ would be expected to increase, because nitrification and denitrification enrich the remaining N (Högberg 1997). The total amount of N deposition these forests have received is not certain, but we estimate 600 kg N ha^{-1} over a 100-year-period (17% of ecosystem N), calculating backward from the current value ($8 \text{ kg ha}^{-1} \text{ y}^{-1}$; Ollinger and others 1993), combined with the observed increase in total N deposition of $0.026 \text{ kg ha}^{-1} \text{ y}^{-1}$ during the last century (Bowen and Valiela 2001). Presumably all of these sites would have experienced this estimated deposition, however the oldest forests would have sustained capacity to retain these inputs over time whereas farm areas would not. Our $\delta^{15}\text{N}$ data suggest that although conventional N accounting measures did not reveal accumulation of atmospheric inputs, deposition inputs are incorporated into the ecosystem.

The modeling to satisfy N and $\delta^{15}\text{N}$ mass balance over succession estimated a $\delta^{15}\text{N}$ value of ecosystem N losses that is only 0.8‰ depleted relative to soil organic matter. This is much lower than published values of fractionation associated with nitrification or denitrification (Högberg 1997), and specifically for nitrate relative to soil N in New England (Pardo and others 2002, 2004). The $\delta^{15}\text{N}$ of DON relative to soil organic N is not well studied, but the fractionation associated with DON forma-

tion may be considerably lower than for nitrification or denitrification, on the order of 1‰ (Houlton and others 2006). Our modeling indicated that the fractionation associated with ecosystem N losses are in this range, and raises the possibility that DON could be the dominant N loss vector over the course of a century in these forests, even during this period of elevated N deposition. The predominance of DON is not surprising given existing N leaching loss patterns for pine forests in New England (Currie and others 1996), and may contribute to the lack of whole-ecosystem N accumulation observed across our chronosequence.

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